A Guide to the Amerithrax Documents: I. Amount of Attack Materials and Requirements for Production

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Abstract:

A review of 2,750 pages of recently declassified FBI documents from the Amerithrax case is examined with special consideration to the question whether the 2001 Postal anthrax attack material could have originated from the United States Army Medical Research Institute for Infections Diseases (USAMRIID). This assessment is hampered by the redactions present. It is concluded that the amount of spores needed for the attack material was considerable, compared to the stocks usually produced and held at USAMRIID. However, it is found that there were several possible avenues whereby sufficient quantities of virulent *Bacillus anthracis* (*Ba*) of the RMR1029 genomic pattern could have been grown or diverted for use in the attack letters. It also is concluded that there were sufficient facilities and expertise at USAMRIID to allow for the purification and drying of the agent material used in the 2001 Postal attack letters. Suggestions for additional investigations are offered.

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[1] This document represents solely the work of Dr. Furmanski and is not a product of the CACNP Scientists' Working Group as a whole.

A Guide to the Amerithrax Documents:
I. Amount of Attack Materials and Requirements for Production

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Introduction:

On Feb 19, 2010, the US DOJ announced the closing of the Amerithrax case, and released a 96 page summary and 2,720 pages of declassified FOIA FBI documents. The 96 page summary presented its scientific and technical conclusions but offered little elaboration or documentation.

Review of the 2,720 pages of the declassified FOIA FBI documents reveals significant scientific and technical elements had been investigated but that were not addressed in detail in the DOJ Summary. A fuller examination of these elements would appear to be of great significance in the resolution of the 2001 anthrax attacks.

Since the NAS has been charged with investigating the scientific aspects of the Amerithrax investigation, identification of certain specific issues appears warranted.

This paper is an effort to identify one of these issues, and specifically to provide a framework for further documentary investigation to resolve them using references in the FBI FOIA documents. Occasional reference to other documents will be made when appropriate.

This paper acknowledges that the DOJ has concluded that Dr Ivins was solely responsible for the anthrax letter attacks and that he prepared the attack material at his laboratory at the USAMRIID facilities at Ft Detrick MD. It will assume that RMR-1029 was the seed stock of the attack material, pending the NAS study group investigation of this issue. It will acknowledge the DOJ assertion that the attack material was not directly diverted from RMR-1029, based on the trace element findings of silicon, but will examine an alternate hypothesis, because significant amounts of RMR-1029 and other anthrax material at USAMRIID remain unaccounted for.

Acknowledging the DOJ positions is not meant necessarily to accept its conclusion, but to act as a framework to determine what scientific information is available in the FBI FOIA documents to support or disprove this conclusion. This paper will draw no conclusions regarding the guilt or innocence of Bruce Ivins.

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Note on Materials Examined and Redactions:

This paper uses as its primary material the 96 page summary released 19Feb2010, officially available at: http://www.justice.gov/amerithrax/ and the 2,720 pages of DOJ/FBI declassified FOIA documents released on 19Feb2010 and officially available at: http://foia.fbi.gov/foiaindex/amerithrax.htm .

The FBI FOIA documents consists primarily of FBI generated reports of interviews or summaries of ongoing investigations, dating from late 2001 to late 2009. On occasion copies of supporting documents collected as part of the investigation are provided, but frequently they are mentioned but omitted. The material originates essentially exclusively from investigations at the USAMRIID campus or in Fredrick, MD. No materials originating from other facilities which were known to possess RMR1029 or its components, such as Dugway Proving Ground and Battelle, are present in the documents. No scientific reports from any collaborating laboratories are present.

Essentially all of the FBI generated documents are redacted to some extent, sometimes quite extensively. By law, redactions need to be justified by citation of one or more exemptions under the FOIA statute.

It is clear that except for Ivins and two or three other individuals known to be deceased, all individual's names and all gender-specific pronouns, and other identifying information such as addresses and social security numbers have been redacted. These redactions are justified by marginal b6, b7C and b7D notations in the redacted documents, indicating the exemption. Because the FBI reports exist in a typeface that has constant spacing, often the number of letters in a subject's surname can be determined. No attempt to identify the identity of individuals whose names have been redacted will be undertaken in this paper, but this fact will be used on one occasion to strengthen the association between two separate interviews involving the custody of a fermentor at USAMRIID.

There are often redactions involving text or entries evidently not involving only the identity of individuals. Sometimes these are short and can be identified by context as specific locations within the USAMRIID campus and laboratories, though because this type of redaction is inconsistently applied between the BEI and USAMRIID series of files, one can often determine locations by cross-reference. Quite frequently these redactions are noted with the b6, b7C and b7D "personal privacy" exemptions, which seems inappropriate.

Often the redactions are quite extensive, involving most or all of a paragraph. Generally, these carry the 'personal privacy' exemption notations, which seems unlikely. In some cases the redacted material can be reasonably surmised to be of scientific character from the context and the unredacted portions. These redactions may have been made because of security issues at USAMRIID or sensitive technical or national security

issues. Examination of these redacted passages may be of importance scientifically, and the NAS should consider appeal of the FOIA redaction, or address the issue by examination of the original documents by NAS personnel with appropriate security clearances.

There is a larger issue regarding the robustness of the material in the FBI FOIA documents. Although extensive, they are a selection of a much larger archive, estimated to be over 50,000 pages. Many documents that would be of interest are mentioned in the available documents but are not included in them. Also, many documents focus on investigations relating to Bruce Ivins, and these are predominantly dated after mid 2005 when he fell under increasing suspicion by the FBI.

Amount of attack material and requirements for its production.

One example of a statement in the DOJ Summary that requires further scientific and technical elucidation is the statement:

"15. A leading anthrax researcher who assisted the investigation expressed his expert opinion that 100 ml would have been required to create sufficient material to be used in one letter, for a total of 500 ml for the five letters. Nonetheless, we cannot say with certainty how much material was used in the letters."[1]

This unattributed statement is far from rigorous: for instance the attack material in Senate letters was qualitatively and quantitatively distinct from that in the NYC/FL media letters. Moreover, no characterization of the nature of the "500mls" is given: it is not specified whether this relates to original media volume, or a product volume, and if so, after what degree of concentration or purification.

Letter Payload

It would appear that a rational estimate of the weight of the attack material can be made, based on the known concentrations of the attack material and the fact that representative examples of both mailings, the Leahy and *NY Post* letters, were apparently recovered unopened[2]. However, an official statement of the amount of powder in these letters has not been made. The unopened Leahy letter has been reported as containing 0.871 grams[3] but no value for the *NY Post* letter has been made public. The Canadian Letter Threat study determined a letter containing 1.0 gram of powder was a reasonable letter payload because it could not be detected by external palpation[4]. Other estimates of letter capacity have been in the 1-2 gram range.

One can conclude from the DOJ summary and other official characterizations of the letter material[5] that the concentration of the Senate attack material was 2.10 x 10^12 CFU/gm, and the NYC/FL attack material was 4.60 x 10^10 CFU/gm[6]. This is a 45-fold difference.

One can calculate that the Senate mailings would have required at least 2 x 0.871 gms =1.74 gms, or 3.64 x 10^12 CFUs. This estimate will be used in this paper as a reasonable approximation of the amount required. It is recognized that the actual amounts used in the two Senate mailings might be slightly lower if the Leahy letter over-estimated the payload of the Daschle letter, or slightly higher if the Leahy letter had lost significant material in the automated postal handling prior to its discovery and/or if it underestimated the payload of the Daschle letter.

The payload of the "first mailing" of the NYC/FL "media" letters is less certain, firstly because the amount present in the NY Post Letter has not been released, and secondly because the total number of letters sent is uncertain. Only two letters addressed to NYC media outlets were recovered (to Tom Brokaw of NBC, and to the *NY Post*), and a third letter to AMI Media in Florida is also officially assumed to have been sent because of the cases of inhalation anthrax and the extensive contamination at the AMI building. The DOJ summary refers to five attack letters. However, an independent epidemiological investigation has proposed that two additional letters to NYC media outlets may have been sent (to ABC News and Dan Rather of CBS News) because of cases of cutaneous anthrax that developed in an employee and an employee's child at those offices[7].

Assuming an average letter payload of 1 gram, the 'first mailings' to NYC/FL would have required between 3 and 5 grams, or between 1.4 and 2.3 x 10^11 CFUs. The actual amount may be lower if only 3 letters were sent with less than 1 gram each, or higher if 5 letters were sent with more than 1 gram each. **But it appears that the first mailing to NYC/FL Media required at least an order of magnitude fewer spores than the second mailing to the US Senate.**

Production parameters for Bacillus anthracis (Ba) spores used in the mailings

By the standards of anthrax material kept on hand at USAMRIID, the anthrax material used in the letter attacks was large. The approximate 2 grams of pure Ba spores (roughly 3.8 x 10^12 spores) used in the letter attacks exceeded the largest single batch of pure Ba spores that USAMRIID had produced in house (the batch designated RMR1030), which took 13 weeks to produce[8]. An examination of how this much material might have been produced or diverted is appropriate.

Solid Media Ba Spore Production:

At USAMRIID virulent Bacillus anthracis (Ba) spores were occasionally produced by harvest from solid media (Petri dishes), typically Sheep Blood Agar (SBA). However, this method produced spores that were 'clumpy,' and contained vegetative cell and media debris that persisted on the spores despite procedures to purify and concentrate[9]. Moreover, the amount of spores harvested per Petri dish was apparently

small compared to those available from liquid culture. No data on spore yield per plate is available in the FBI FOIA documents for calculation, but it was stated by Ivins that production of the letter attack material from solid media was impractical because of the large number of Petri dishes that would have been required[10].

However, plates containing virulent Ba were apparently abundant at USAMRIID. FBI FOIA documents indicate each aerosol challenge trial in building 1412 at USAMRIID produced approximately 180 Petri dishes growing RMR1029 per day and such challenge trials were often performed three days a week[11]. These Petri dishes and other materials containing Ba from multiple challenge tests were frequently allowed to accumulate in the containment area and to grow-out for many days or weeks after examination and before the infectious waste was autoclaved[12]. The FBI calculated that the accumulated post-challenge material after aerosol testing for 3 or more animals would have been sufficient to create the letter fill material[13], though the calculations for this determination are not explained or provided. Since at most only about 24 ml of RMR1029 challenge material would be issued for a 3 animal test, and the letters would have required at least 100 ml of undiluted RMR1029 (see below) , the bulk of the requirements for the letter fill under this estimation would have been accounted for by the overgrown assay plates in this scenario.

The FBI devoted considerable efforts to determine if this material might have been diverted either in bulk to make the attack material or in smaller amounts to act as seed for further culture[14]. This investigation revealed faults in the automated security system in building 1412, specifically that the key-card security system in building 1412 showed system faults and/or evidence that individuals 'piggy-backed' through the control points in about 50% of the cases, but concluded that access to the internal containment area could probably be determined for each entrant[15].

In addition, it appears that on occasion 'hot' trash was not autoclaved before being removed from the containment area, and remained in the basement storage area before being autoclaved before leaving building 1412[16]. This basement area was also considered 'hot' but its access was apparently controlled by a single keycard access point and maintenance as well as technical/professional employees had access. The FOIA documents do not present a detailed investigation of the security of this area.

It appears that on occasion 'anti-foam' agents were added to the aerosol generator in the aerosol challenge apparatus in building 1412, and the FBI undertook extensive questioning of USAMRIID employees about the frequency this occurred and the antifoam agents utilized. Due to redactions, it is impossible to determine if silicon containing antifoam agents were used, and, if so, in what amounts and how frequently. No data is present in the current FBI FIOA documents regarding if silicon containing antifoam agents might influence the silicon content of the Ba organisms plated from the AGI material.

Large numbers of agar plates were handled in the main bacteriology division laboratories in building 1425 as well. Single series of experiments could routinely generate hundreds of plates containing Ba[17].

Liquid Flask Ba spore Production Parameters:

At USAMRIID virulent Bacillus anthracis (Ba) spores were routinely produced by harvest from solid media or by liquid media in shaker flasks. For significant amounts, the shaker flask method was used because it produced a larger quantity of cleaner spores than solid medium.

The standard production run at USAMRIID was made with 2-liters of liquid Leighton and Doi medium, shaken in eight 250 ml aliquots in eight flasks. Good objective data on the yields of this method are available in documents recording the production of RMR1030, a batch of concentrated Ba spores used for aerosol challenge tests in the late 1990s, and produced by 2-liter liquid media batches at USAMRIID, in Ivins' lab. Yields were variable and not predictable, with 10% to 20% unsatisfactory runs produced even by experienced technicians including Ivins himself[18]. Unsatisfactory runs were discarded. Some 2 liter flask runs yielded after gradient purification as much as ~ 3.5 to 4.0 (3.46,3.97) x 10^11 CFUs per 2 liter run, but based on a larger sample of 'acceptable' runs, (the yield of 13 individual runs that produced RMR1030), the average yield was 2.3 x 10^11 spores per 2 liter run.[19]

Based on these numbers, if the Senate letters originated from 2-liter liquid medium runs, it would take about 16 (sixteen) successful 2-liter runs. These runs would have required a total of 32 liters of starting medium, and would have taken 16 to 19 weeks at the usual rate of one run per week, depending on how many unsatisfactory runs occurred. After purification liquid flask produced spores could be concentrated to any desired volume, but they were typically stored in phenol in concentrations ~1.3 x 10^10 cfu/ml (ie RMR 1030)[20]. **The Senate letters would have required 277 mls of purified, concentrated liquid flask produced spores, such as RMR 1030.** RMR1030, the largest pool of liquid medium spores produced at USAMRIID, was originally only 250 ml in volume.

If the NYC/FL letters originated from 2-liter liquid medium runs, it would take one 2-liter run (~2.3 x 10^11 spores).

On several occasions Ivins addresses spore preparation and the requirements for the attack letters in interviews with the FBI. These are rough estimates, based on 2.0 to 2.5 grams per letter, and do not specifically address the 45-fold difference in concentration between the NYC/FL and the Senate letters. He concludes that it would take ~ 100 liters of liquid medium culture or ~ 300 ml of "Dugway material" (ie RMR1029) to fill the five letters[21]. These 'ballpark' estimates were echoed by another USAMRIID worker[22].

Fermentor production of Ba spores:

Ba Spore production at Dugway Proving Grounds (DPG):

Dugway Proving Grounds (DPG) produces Ba spores by fermentor. Standard Operating Procedures (SOPs) sent to USAMRIID for RMR1029 indicated they were produced in a counter top fermentor 10 liters or less in capacity[23]. Ivins recalled that the DPG fermentors were 8-10 liter capacity[24]. The DOJ summary refers to "12 ten-liter fermentor grown lots.[25]" Ivins' logs for the production of RMR 1029 are heavily redacted, but from the available data, each fermentor run at DPG could produce at least 3 x 10^12 total spores with some runs achieving 5 x 10^12 spores[26] before processing at USAMRIID. Due to redactions, the yield after processing at USAMRIID is uncertain, but one 5 x 10 ^12 run apparently produced 3.8 x 10^12 cfu after processing (76% yield)[27]. The average yield per DPG run after processing cannot be determined because of the redactions.

Therefore enough Ba spores for the two Senate letters might have been produced by a single production run of a counter-top fermentor of the size used at Dugway.

Dugway spores were sent to USAMRIID to provide challenge material, beginning in 1997 for the creation of RMR 1029. Seven shipments transferring a total of 13 production runs were sent to USAMRIID. The final (7th) Dugway shipment, containing the single 13th production run was received at USAMRIID in 1997 but was not included in the final RMR1029 product because Ivins reported it could not be purified to an acceptable degree. Ivins reported he had 'set it aside' for destruction by autoclaving, but could not recall actually doing so, nor were records kept of such destruction[28]. It was apparently common practice to retain samples of spores indefinitely at USAMRIID, so is possible that this material might have been retained and been available in 2001.

It is doubtful that this final production run of spores from DPG could have been processed into the Senate letter attack material, even if, after having been found unsatisfactory for incorporation into RMR 1029, it could have been processed to the high concentration and purity of the Senate attack material. It is uncertain if would have had the polymorphic genetic signature of RMR1029, because it is not clear if this polymorphism was due to polymorphism in the seed culture used in the fermentors at DPG, or if (as seems more likely) it was the result of the admixing of multiple runs of DPG material with multiple single-colony origin 2-liter batches of Ba Ames grown at USAMRIID. It would have a Carbon-14 (C-14) profile older than that reported for the attack material, which was officially reported as placing the age of the attack material as within 2 years of late 2001[29] (ie it would have had to have been grown after late 1999). However, the ability of the C-14 data to exclude dates of production in the 1997-1999 range may not have been robust, because the C-14 data, known in late 2001, did not prevent the FBI investigation from pursuing the possibility that RMR1029 itself may have been directly diverted to constitute the attack material as late as mid 2004[30], during a period when Steven Hatfill was suspected of diverting RMR1029 from the aerobiology building waste. It may also be significant that the 2010 DOJ Summary does not include

the C-14 data in its scientific characterization of the attack materials. A clarification of the significance of the reported C-14 data should be undertaken.

Although no sample of Ba spores yet reported had a silicon content as high as the attack material, the highest value reported was from a Dugway fermentation Ba product, so the 13th batch may have had a high silicon content.

Fermentor production of Ba at USAMRIID

USAMRIID had several fermentors in the Bacteriology Division labs in Building 1425, though the exact location is unclear because of redactions in the FBI documents and the lack of a floorplan for reference. It appears that they were located in or adjacent to the B3 suite BSL-3 containment facility[31].

USAMRIID workers did not produce virulent Ba spores in these fermentors, because it was considered too dangerous in a metropolitan area[32]. These fermentors were used for producing attenuated strains of Ba and nonpathogenic organisms.

Although equipment records are apparently incomplete, there were several fermentors of varying sizes at USAMRIID in 2001, including 150-liter, 20-liter and 5-liter New Brunswick models. The 150-liter model occupied a dedicated room and was large, noisy, and could not have been operated in an unobtrusive fashion[33]. The 20-liter New Brunswick fermentor had been in use in 2000 in a vaccine project, which apparently did not involve Ivins[34]. Little other information on this 20-liter fermentor appears in the documents.

Considerable information is available regarding the 5-liter New Brunswick Bio-Flo 3000 fermentor.

This Brunswick Bio-Flo 3000 was a unit that had been acquired by USAMRIID in Jan 1990 and was on Bruce Ivins' personal 'hand receipt' indicating it was purchased for use by his laboratory team and was under his direct control[35]. This fermentor was used by Ivins in the 1990s to grow genetically modified organisms for the production of the Recombinant Protective Antigen (rPA) anthrax vaccine that was his major professional endeavor at USAMRIID. This fermentor is mentioned specifically in his patent for the rPA vaccine[36]. The patent specifies that antifoam C was added to the culture medium[37]. Antifoam C is a commercial antifoam agent manufactured by Dow Corning and contains silicone[38]. In the rPA vaccine work, Ivins did not use this fermentor to produce spores, since the genetically modified Sterne strain used to produce Protective Antigen (PA) for the rPA vaccine was non-sporogenic[39].

However, an entry in one of Ivins' laboratory notebooks seems to indicate he did use this fermentor to attempt to produce virulent Ba spores on at least one occasion [40]. Prior to the 2001 anthrax attacks, Ivins was the technical representative to DPG for the project where DPG produced virulent Ba Ames spores for RMR1029[41], and Ivins received the protocols for production of virulent Ba Ames in small fermentors[42].

The 5-liter Brunswick fermentor produced little noise when running, and standard operating procedures for fermentors at USAMRIID included the automatic addition of Mazu, an anti-foam agent into the fermentors[43]. Mazu is a silicon-containing anti-foam agent[44].

The physical location of this 5-liter fermentor just prior to the 2001 letter attacks was investigated but due to extensive redactions in the available record, it is unclear what the FBI determined. It appears that Ivins reported he had lent the instrument to an entity whose identity is redacted (presumably another investigator at USAMRIID) on an undisclosed date[45]. It appears that the reported recipient of the instrument may have denied ever having possession of it[46]. The location of this fermentor, its condition, and its capability of producing the attack material in late 2001 should be clarified by a suitable examination of the unredacted FBI record.

Assuming that the final concentration that the 5-liter fermentor at USAMRIID could achieve was proportional to that of the 10 liter fermentors at DPG, it might produce ~ 1.9 x 10^12 spores per 5-liter run.

Therefore enough material for the Senate letters might have been produced in two runs of the 5.0 liter fermentor at USAMRIID.

Diversion of USAMRIID Ba for attack culture seed

Prior to fall 2001, considerable amounts of Ba (hundreds but not thousands of containers) were stored at USAMRIID without inventory control in common storage areas accessible by all authorized workers at USAMRIID[47]. Also, Ba challenge material, including significant volumes of RMR1029, which were normally held at the main USAMRIID building, 1425, was regularly transferred to the aerosol test building 1412, for use in aerosol testing. These transfers consisted of concentrated or diluted RMR1029 material and there were significant unused portions of the challenge material that remained in building 1412 for extended periods of time under imperfect security. This has been discussed above in the section of agar growth of Ba spores.

Essentially all USAMRIID employees stated that, prior to the 9-11 attacks, it would not have been difficult for someone who worked at USAMRIID to clandestinely remove viable virulent pathogens from the containment labs and transport them off of the USAMRIID campus.

Diversion and alteration of RMR1029 for attack material

This paper will now examine the opportunities to divert significant proportions of RMR1029 for direct processing into the attack material. This is because very significant amounts of RMR1029 and associated USAMRIID "wild type" Ames Ba spores appear to be unaccounted for.

The DOJ contends that the attack material could not be a direct diversion of RMR 1029 itself because the attack material contained a high percentage (65 to 78%) of spores with high silicon content in the spore coat and RMR1029 showed essentially none (0 of 300 spores examined)[48]. The report contends that this difference is due to growth of low-silicon containing RMR1029 seed material in a cultural environment that resulted in the incorporation of high levels of silicon into a high percentage of the spores in the attack material. Incorporation of silicon into the spore coat from growth medium is a process that has been shown to occur in Bacillus species including Ba. The identification of silicon in spores forming within vegetative cells in the attack material strongly supports the contention that the silicon was incorporated from the culture medium[49].

This scenario has two weaknesses. The first is that it does not quantitatively explain either the very high percentage of high-silicon spores in the attack material, nor the apparent absence of high-silicon spores in RMR1029. Despite efforts to duplicate the attack material, no preparations of over 30% have been made or found in collected samples of Ba spores. Several samples of spores from DPG have been reported as containing from 11% to 29% high silicon spores. RMR 1030, a preparation pooled from thirteen 2-liter liquid cultures of Ba grown at USAMRIID contained 6% high silicon spores[50]. Since RMR1029 was originally pooled from both DPG and USAMRIID material in approximately a 85%/15% ratio, one would have expected it should have had ~ 20% high silicon spores (range perhaps 10% to 25%). Even if the DPG component of RMR1029 had no high-silicon spores, the USAMRIID component should have contributed ~ 1% to the final mixture, and been detectable in the 300 spores examined.

The other weakness is that a sample of RMR 1029 suitable for silicon determination was apparently not obtained from USAMRIID by the FBI until 3Jun2004, and so may not have been representative of RMR 1029 in 2001 when the attack material was prepared.

An alternative explanation for the wide discordance in silicon content between the attack material and the 2004 RMR1029 sample is that the original RMR1029 had a moderate percentage of high-silicon spores (10-25%) and in 2001 underwent a manipulation that selectively enhanced high-silicon spores in the attack material and depleted them in the residual RMR1029 container. Such a differential purification may have been the unintended result of decanting or pipetting from a sedimented RMR1029 flask, or the result of centrifugation and division of a stratified pellet. Centrifuged pellets of Ba are known to demonstrate a banded appearance.

A discussant at the 25Sep2009 NAS meeting suggested that graded manipulation of the number of high-silicon spores in a sample might be possible because a series of samples designated “evidence” showed progressive results from 18% to 1.2%[51]. This series and any other similar data should be investigated for applicability to this hypothesis.

The perpetrator may also have subjected the original RMR 1029 material to more sophisticated, deliberate manipulations, such as additional gradient purifications, to introduce ‘red herrings’ in the trace materials left on the Senate attack material. The perpetrator was skilled in avoiding leaving forensic traces (there were no significant hair,

fiber or human DNA traces on the letters) and the NYC/FL material was clearly left crude (and possibly deliberately contaminated with *B subtillis*) to mimic an amateur's efforts. It may be significant that Ivins spontaneously suggested to the FBI that they look for traces of gradient material in the attack material[52]. A novel manipulation may have inadvertently differentially separated the silicon bearing spores. An inquiry into what trace materials were found in the attack material should be made.

As long as a residual fraction of the original RMR1029 pool remained, the genetic polymorphisms that defined the qualitative genetic signature of RMR 1029 would be retained in both components despite an alteration of the profile of the silicon in the spore populations.

It should be noted that RMR 1029 was maintained at a concentration of ~ 2.5 to 3.9 x 10^10 cfu/ml[53], and therefore would have required at least ~ 93-146 ml to be used to produce the material in the two Senate letters, and perhaps more if the process was inefficient or had failed runs. Although the inventory of RMR 1029 provided by Ivins to the FBI is untrustworthy, it lists the volume of RMR1029 as being 369 ml prior to the 2001 letter attacks[54], though it may have been 100 ml more because of an arithmetical error. Withdrawal of one or more 100-150 ml aliquots to prepare the attack material could have greatly depleted the component of high-silicon spores in the residual RMR1029 if that 100 ml aliquot was highly enriched in high-silicon spores.

The above speculations would not be particularly compelling except that a very significant amount of RMR 1029 is unaccounted for, and significant amounts of 'wild type' Ames grown at USAMRIID are also unaccounted for.

The inventory record of RMR1029 was imperfect. Ivins had created RMR1029 and was its custodian. He initially stated in early 2002 that he kept precise records of its creation and distribution and that it was completely accounted for[55]. However, the log of RMR1029 distributions he provided to the FBI in February 2003[56] contained a 100 ml arithmetical error logged in a 22Feb2000 distribution that resulted in 100 ml of RMR 1029 being unaccounted for in this log. Later, Ivins maintained that the RMR 1029 distribution log was only an estimation and not a measured inventory[57].

This discrepancy resulted in an FBI investigation to trace the distribution of RMR1029 by cross-checking lab notebooks of the recipients of RMR 1029 with Ivins' log. This determined that at the time of the 2001 mailings there was a total of 220 ml of RMR-1029 that was unaccounted for[58]. This investigation is summarized in the DOJ summary but only fragmentary and redacted portions are present in the current collection of FBI FOIA documents, wherein Ivins addresses some of the specific inconsistencies by invoking evaporation and stating in some instances he centrifuged and concentrated RMR1029 before shipping to Battelle[59].

Based upon the distribution log and the FBI investigation of distribution of RMR1029, there is a sufficient amount of RMR1029 unaccounted for at the time of the letter attacks to supply the attack letter material.

If a substantial portion of the original RMR1029 had been diverted to produce the attack material in late 2001, Ivins would have potentially faced a significant shortfall of challenge material for the challenges anticipated for late 2001, 2002 and 2003, for which RMR1029 had been prepared. In fact, Ivins sometime in 2001 requested additional spores be obtained from Dugway[60]. According to Ivins' log of RMR1029, and correcting for the 100 ml arithmetical error, there should have been 469 ml of RMR1029 remaining at the time of the letter attacks in Sep/Oct 2001, nearly half of the original 1,000 mls. A determination of the date and examination of the justification of this order for DPG spores should be made.

Perhaps pertinent to this point, a further FBI investigation revealed that essentially all of what must have been a very substantial amount of Ba spore production by Ivins' two technicians at USAMRIID was also unaccounted for[61]. Both of Ivins' technicians thought that their Ba spore production was to augment or replace an exhausted or dwindling supply of RMR1029, and one understood she had been hired primarily to make spores.

In the 16 months between the letter attacks and the end of the RMR1029 log, Ivins records a total of 281 mls of RMR1029 released. The 281 mls of RMR 1029 would amount to ~ 10^13 spores, which could be produced by 43 successful 2 liter liquid cultures of Ba, perhaps a year's production of Ivins' lab, considering the 10-20% rate of unsatisfactory cultures. It may be significant that the spore production in Ivins' lab may have just been sufficient to meet this demand.

The DOJ summary states that RMR1029 never was modified from its creation by additions[62], though it offers no supporting evidence. The above scenario does not require additions to the RMR1029 flask, though additions to it of cultures containing few or no high silicon spores would dilute any residual concentration of high-silicon spores. Perhaps significantly, there is an episode in the FOIA documents that suggests that RMR may have been diluted and Ivins may have been altering RMR1029 by adding spores as late as mid 2004.

The FBI obtained the entire RMR1029 flask on 3Jun2004 and it containing about 75 ml[63]. The Naval Medical Research Center (NMRC) determined that its concentration was approximately tenfold lower than Ivins' log stated it should have been (~10^9 instead of ~10^10 cfu/ml). A 25 ml portion of RMR1029 was then returned to Ivins for continued use at USAMRIID, and the remainder retained at NMRC[64]. Apparently the FBI informed him of the low count from NMRC on 13Jan2005. On 16Feb2005 Ivins replied that he had tested the returned portion, and that it was only about 20-30% less concentrated than usual, and the FBI report related that Ivins stated that it was not a problem because they added more spores for the aerosol challenge[65]. While this is apparently an admission of adding spores to RMR1029, Ivins later denied he added spores to the returned RMR1029 material. Since challenge material was typically diluted from RMR1029 before use, the FBI report may have misinterpreted Ivins referring to a lower dilution factor as a physical addition. Or Ivins may have been untruthful. The FBI confronted Ivins with the discordance in concentrations more forcefully on 31Mar2005,

and suggested that a significant amount of RMR1029 might have been diverted and replaced with water. He denied adding spores to the returned RMR1029 material, and suggested the NMRC concentration value was in error. Ivins stated that since its return, he had provided 25ml in a successful animal challenge test, which would indicate it was not 10^9 cfu/ml[66]. Ivins' RMR1029 stock was not exhausted, however, because Ivins and an FBI agent (apparently a trained microbiologist) immediately determined the concentration of Ivins' RMR1029 stock in parallel assays, and confirmed Ivins' higher concentration[67]. The FBI agent secured Ivins' residual RMR1029 stock at this point. Further documents reveal additional studies were planned on all available samples of RMR1029 to resolve this issue, but no resolution is presented[68].

Ivins stated in 2008 that the FBI accused him of diverting and/or altering RMR1029[69].

Another factor to be considered is that Carbon-14 data from the attack material was reported as indicating it had been grown within 2 years of 2001. As discussed above in the context of the unused 13th batch of Dugway spores, the ability of the C-14 data to exclude origin in 1997-1999 needs clarification.

Drying of Spores

The attack spore material was dried and the Senate attack material was fine enough to be spontaneously aerosolized by the opening of the letter in Senator Daschle's office. The DOJ summary states that the spores had a mass median diameter of between 22 and 38 microns, indicating that they commonly did form aggregates when aerosolized (individual spores are barrel shaped, roughly 1 x 1.5 microns). The DOJ summary reports no indication of special treatment or 'weaponization' was identified[70].

Dry spores at USAMRIID

Ba spore material was very seldom dried at USAMRIID. Rare instances of drying very small amounts of virulent strains of Ba were noted, but these were dried only after sterilization by radiation, and were for antigen production for in vitro test systems[71]. A highly redacted entry suggests live Ba might have been dried in very small amounts[72].

When it was reported that Dugway Proving Grounds (DPG) produced dry, virulent Ba spores, that fact surprised many USAMRIID employees. There is no direct information regarding the DPG spore drying process or program in the FBI FOIA materials.

USAMRIID employees reported that it was thought that Battelle did work with dried Ba material[73], but no details were offered and no additional information is present in the FBI FOIA documents regarding Battelle.

USAMRIID had contemplated initiating aerosol challenges using dry, virulent Ba spore preparations, and this project had proceeded to initial laboratory work using dried non-pathogenic organisms, but the program was terminated before any dry virulent Ba spores

were handled. The reason for the termination was stated by some USAMRIID employees as being 'dual use' concerns under the Biological Weapons Convention (BWC) [74], but others offered different [redacted] explanations[75]. There is no technical information available on the proposed method of drying or equipment used in this initial program, but it apparently some equipment was obtained and some activity took place in the aerobiology building, 1412, rather than at the main bacteriology department in building 1425. This may have been a project of the Aerobiology Division rather than the Bacteriology Division of USAMRIID.

Ivins' knowledge of this program, that contemplated use and possibly production of dry virulent Ba spores at USAMRIID, is unclear from the FOIA record. Interviews regarding the history and organization of this project are highly redacted, but Ivins name, which generally is not redacted, does not appear in these documents. When asked he denied any knowledge of it[76]. However, one of the USAMRIID employees who described the project at length stated it was initiated to test the 'breakthrough point' of the new rPA vaccine, and that discussions had addressed the options of obtaining dry Ba spores from DPG or producing them in-house at USAMRIID. Although Ivins was an employee of the bacteriology division rather than the aerobiology division of USAMRIID, he was one of the essential personnel involved in aerosol Ba challenges at USAMRIID, providing the challenge Ba material and personally participating in every challenge in the Aerobiology Division building 1412. Moreover, the new rPA vaccine was his personal career project, and he was the technical liaison for spore procurement between USAMRIID and DPG. A CDC informant apparently informed about both DPG and USAMRIID procedures assumed that Ivins had knowledge about DPG's methods to dry Ba spores[77]. A further exploration of Ivins knowledge base on drying Ba spores should be undertaken.

Methods of Drying Spores

There are a variety of potential methods to dry Ba spores. Some apparently can be done with minimal equipment, others require specialized instruments. Industrial scale drying is often done with spray-dryers, but no spray-drying apparatus was present at USAMRIID. Attention has focused on instruments called lyophilizers, which might be used to dry Ba spores, and were present at USAMRIID.

Lyophilizers at USAMRIID

Lyophilizers are also termed 'freeze dryers' and classically operate by freezing a sample and then allowing the water in the sample to sublimate away under a vacuum and be trapped in an adjacent 'cold trap' where the water vapor is again frozen. Lyophilizers are often used in this mode to preserve some bacterial cultures and other biological samples. However, Ba spores are damaged by freezing. Many large lyophilizers can be adjusted so that the sample compartment is kept at any desired temperature, and the liquid water vapor evaporated under reduced atmospheric pressure and trapped in an adjacent cold trap. This mode might better be characterized 'vacuum drying'.

Two types of lyophilizers were present at USAMRIID. There were many small units generically termed 'Speed-Vacs' which are counter-top instruments for lyophilizing small volumes of material. Typically they can accommodate multiple small tubes holding 2 milliliters or less, freeze the contents and subject them to centrifugation under vacuum. Because it is likely that the wet spore aliquots used to create the Senate letter fills would have been at least 50 or 100 ml in volume, were not frozen, and because centrifugation presents a significant aerosolization hazard, the use of Speed-Vacs has generally been discounted.

The other type of lyophilizer is a large, batch lyophilizer capable of containing and evaporating several liters of fluid in a single run. Present in the USAMRIID bacteriology division was a Vertis 12XL "Freeze Mobile" Condenser Trap Unit equipped with an attached Vertis HL600 3-shelf UniTop specimen chamber. This is a mobile one-piece unit mounted on wheels and is the size and general appearance of a 'stacked' clothes washer/dryer appliance. The specimen compartment can contain up to 15 liters of fluid to be dried in 125 ml bottles, and it temperature can be independently controlled. The condenser is capable of evaporating ~8 liters of fluid in 24 hours.

This large Vertis lyophilizer was on Dr Ivins' 'hand receipt' indicating it had been purchased for his laboratory in 1996 and was under his control. It was apparently kept in the hallway between the B3 BSL-3 containment suite and the B5 BSL-2 general bacteriology laboratory in building 1425. Ivins had used it in the rPA vaccine project in the 1990s to lyophilize non-viable biological products, and was trained and experienced in its use.

Conclusions

There appears to have been sufficient resources at USAMRIID to account for the production or diversion of sufficient material for the letter attack payloads. The NYC/FL letters might have been grown in a single, routine 2 liter batch at USAMRIID without attracting any attention. The Senate material, although requiring an order of magnitude more material, had several potential sources. A fermentor apparently existed in the USAMRIID lab that was capable of growing the attack material in two production runs. Unmonitored RMR 1029 waste from aerosol challenge tests in building 1412 was sufficient in quantity to be diverted into the attack material, and was not tightly secured. There was sufficient material unaccounted for in the RMR 1029 flask itself to account for the Senate attack materials, and sufficient Ba Ames material unaccounted for in the USAMRIID lab to account for the shortfall from such a diversion.

There appears to be adequate facilities for the drying of the attack material. It may be that little equipment would be required outside of normal laboratory facilities, but a large capacity versatile lyophilizer was present that might have been used.

Suggestions

1. Official confirmation of the amount of letter material in the Leahy letter and *NYC Post* letters should be sought.

2. Examination of the record for scientific data relating to chemical traces on the attack material, such as residua from residual media, gradient purifications, or effects of drying methods. Any trace residua should be correlated with established procedures and available reagents at USAMRIID and other laboratories of significance.

3. The results from (2) above should be correlated with the corresponding results from FBI sponsored attempts to duplicate the letter fill materials.

4. Further elucidation of the details of the quantitative distribution of high-silicon spores in various samples of interest, such as the attack materials, and the several samples of RMR1029 itself. Particular attention ought to be made to procure as wide a selection of samples as possible to determine sampling errors.

5. Similar quantitative determinations of the incidence of high-silicon spores be made on the silicon distribution in representative samples of Dugway fermentor and USAMRIID 2-liter batches of spores.

6. Investigation of the possible differential partition of high and low silicon spores by various methods such as sedimentation, centrifugation, or division of centrifuged pellets. The origin of the graded series of Dugway materials alluded to in the NAS presentation be determined for its relevancy to this issue.

7. The confidence values for C-14 age of the attack material and of other significant materials, such as RMR 1029 should be determined and clarified. The significance of this data to the forensics of the attack material should be clarified.

8. The operational status and location of the 5-liter fermentor in late 2001 should be determined and clarified.

9. Location and operational status of the large Vertis 12XL ‘Freeze Mobile” in late 2001 be determined.

10. The alleged ‘dilution’ of RMR 1029 in mid 2004 be resolved by a full examination of the record.

11. A review of the records of production of Ba Ames strain by Ivins’ technicians to determine the amount of unaccounted for Ames and the dates of its production.

12. A determination of the date and examination of the justification for Ivins’ 2001 order for additional DPG spores should be made.

13. A review of information regarding Ivins' knowledge of Ba drying techniques, and particularly his contact with the dry-spore challenge program at USAMRIID should be made.

ENDNOTES

[1]DOJ Summary, Footnote 15, page 29 (PDF page 33)

[2] UCLA Department of Epidemiology School of Public Health, Bioterrorism website:
"Exposure Letters" states the *New York Post* letter was handled but not opened: accessed 11Apr2010 at: http://www.ph.ucla.edu/EPI/bioter/detect/antdetect_letters_a.htm

[3] Broad WJ and Johnston D. "Anthrax Sent Through Mail Gained Potency by the Letter" NYTimes 7May2002.

[4] Kournikakis B et al. Risk Assessment of Anthrax Threat Letters. Defence R&D Canada Technical Report DRES-TR-2001-048 Sept 2001. Available online at: http://www.anthraxinvestigation.com/canadiananthraxstudysep01.pdf

[5] United States Department of Justice Science Anthrax Press Briefing, Monday August 18, 2008. Transcript accessed 11 April 2010 at: http://www.fredericknewspost.com/media/pdfs/FBI_0818_afternoon_bfg.pdf

[6] DOJ summary pg 14 (PDFpg 19).

[7] UCLA Department of Epidemiology School of Public Health, Bioterrorism website:
"Exposure Letters: Seven Letters Sent" gives details of this hypothesis: accessed 11Apr2010 at: http://www.ph.ucla.edu/EPI/bioter/detect/antdetect_letters_a.htm

[8] USAMRIID #16 PDFpg 73, 74: FBI review Ivins' lab notebooks 15May2004: summary of Jan 1997 entry: *"The current batch of Ames spores (RMR 1030) took 13 production runs…He noted that 13 runs had yielded 3 x 10^12 spores."*

[9] BEI#1 PDFpg4: 23Jan2002 interview with Bruce Ivins:
"..the spores in the DASCHLE letter were of fermentation quality. The spores were very clean compared to Bacillus anthracis produced on agar which would contain messy residue."
BEI#1PDFpg24: Ivins interview 12Feb2003:
"IVINS thinks that most of the people at USAMRIID use broth to grow Ba although he can't say for certain how everyone else there grows their spores. Although Ba can be grown on agar, nicer spores are produced in broth, and they can be purified more easily and are "hotter". ...
Spores grown up on agar are dirtier than broth grown. IVINS does not know if this is because the spores pickup debris from the agar of if there are media components on the surface of the spore. No matter how agar grown spores are washed, some agar always remains on the spores."
BEI#2PDFpg 61: 1Mar2007 Interview with USAMRIID employee:
"SBA [Sheep Blood Agar] leaves blood residue on the spores, and liquid spore preparations are easier to clean."

[10] BEI#1PDFpg24: Ivins interview 12Feb2003:
"If one needs to purify more than a very small amount of Ba, it is difficult to use agar as you would need numerous plates."

[11] USARMIID#15 PDFpg 15: Interview with USAMRIID employee, 9Mar2004:
"Challenges were run three (3) days per week with thirty (30) rabbits per day. Two (2) dilutions of anthrax were prepared per rabbit for each challenge, and three (3) Tryptic Soy Agar (TSA) plates were cultured per dilution. [redacted] noted that a total of approximately 180 TSA plates were cultured per day when animal challenges were being conducted."

[12] USARMIID#15 PDFpg 16: Interview with USAMRIID employee, 9Mar2004:
"After the plates had been left in the incubator overnight, they would be completely covered with growth. The plates were then read in the morning following the challenge. After the plates were read, they were placed into a bag and clearly marked with what pathogen was in the bag, what strain the pathogen was, and who the investigator was. The bags would remain in room [redacted] until nearly overflowing, or until the number of bags in the room became an obstruction. The bags often sat in room [redacted] for several days or weeks prior to being removed. [redacted] noted that [he] was fascinated with how much growth appeared on the plates after several days or weeks. The bags were then taken to the basement to be autoclaved."
USAMRIID#8 PDFpg 77: Washington Field Office Summary Report 11Jan2006:
"It was documented during several interviews that Ivins' group did not keep room [redacted] very clean and tidy. Post-challenge agar plates were left on counters, the incubators were left full of material, samples in the refrigerator were not disposed of in a timely manner, and "hot" trash was allowed to build up for weeks prior to being autoclaved. One former military aerobiology technician [redacted] commented that [redacted] had to clean Ivins' trash himself out of safety concerns. [redacted] said that the civilians at USAMRIID did not take safety seriously. [redacted] commented that when [redacted] looked at the agar plates that had sat in the biohazard trash bags for several days or weeks in 115, they were covered with bacterial growth."

[13] USAMRIID#20 PDFpg 30: 20May2004 Report investigative project re access to post-challenge Ames from Aerobiology Division USARIID:
"It was estimated based on spore concentrations of material used that any aerosol challenge involving greater than or equal to three animals would constitute enough available Ba to have assemble the anthrax-laced letters."

[14] BEI#1 PDFpg 45: 17 April 2003: telephonic interview with Bruce Ivins:
"After his telephonic conversation with SA [redacted] on 4/15/2003, IVINS feels sick over the fact that the material used in the anthrax mailings cold have come from a stock made from the BA aerosol challenge trash."

[15] USAMRIID#4 PDFpg16: Summary of results of initiatives, 29Mar2006:

"For all of the days when access points were recorded for employees who used the containment area to work with aerosol challenges, at least three of the five access points were recorded together. There was therefore always some indication that the employee used the change room or keypad to enter the containment area. In approximately 50% of the cases, one or two of the access points were missing (ie 3 of 5, or 4 of 5 points recorded). ... Never on a day when an employee was assigned to work in the containment area was he/she shown only to have entered and exited the building on that day."

USAMRIID#4 PDFpg20: Summary of results of initiatives, 29Mar2006:

"Anomalies indicate that the employee may have piggybacked through a missing access point with another individual, or the system might not have read a keycard swipe of keypad code properly."

"The amount of anomalies evident on the spreadsheet imply that there were most likely incidences of piggybacking and/or system faults through use of the change rooms into the containment area of Building 1412 on these dates."

"The initiatives described above demonstrate a significant difference between expected keycard access data for employees using the containment area of Building 1412, and the actual data for employees during several Ba aerosol challenges between 9/1998 and 8/2001."

[16] USAMRIID#8 PDFpg81: Washington Field Office Summary Report 11Jan2006:

"Information obtained from interviews indicated that standard protocol is and was for the post-challenge plates to be autoclaved in the challenge labs prior to disposal in the basement, meaning that this material should have been autoclaved twice. Several technicians stated that this was not always the practice. All material on the hot side of building [redacted] was considered to be 'hot', and it was the opinion of several technicians that the PIs and their staff were not concerned if material was autoclaved before leaving a room or lab for disposal. The main concern was that material was autoclaved in the basement before leaving the hot side of the building."

[17] USAMRIID#15 PDFpg 45: Interview with previous USAMRIID employee 6Apr2004:

"There could have been large numbers of agar plates in labs at the given time without sending up a red flag. In serial sacrifice experiments, a group of animal was exposed to a pathogen. A few animals were removed and sacrificed on a time table to determine the bacterial load over time. [long redaction] Ten-fold dilution were prepared for each organ. Five to seven plates were prepared per organ. They would sacrifice three to five animals per time point, so there were hundreds of plates per serial sacrifice experiment."

[18] BEI#2 PDFpg15.

[19] FBI FOIA USAMRIID #16 PDFpg 73, 74: FBI review Ivins' lab notebooks 15May2004: summary of Jan 1997 entry:

"The current batch of Ames spores (RMR 1030) took 13 production runs…He noted that 13 runs had yielded 3 x 10^12 spores."

Also see USAMRIID#1PDFpg 64 for same info.

[20] FBI FOIA USAMRIID #16 PDF pg 73.

[21] BEI#1 PDFpg 14: (2/26/2002) *"Ivins noted that the ratio of spores to media for B anthracis in a liquid preparation is approximately 10^8 spores per ml. Therefore, it would take 20 liters of media to make two grams of spores."*

BEI#1 PDFpg 24: (2/12/2003) Ivins interview:

"IVINS did the following calculation estimates to determine how much of the Dugway spores would have been missing if they had been used in the anthrax mailings. 2.0-2.5 grams of material were present in each letter with a 10^8 spore concentration. In order to achieve that spore concentration, 80-100 L of runs would have been necessary and approximately 300 ml of the Dugway material would have been required. This amount of missing material would have been noticed." [MF note: this takes above estimate and multiplies it by 4 or 5 for the 4 or 5 letters].

[22] USAMRIID#8 PDFpg 9: 18Aug2005 interview with ex-UAMRIID employee:

"…the individual would have needed to grow hundreds of liters in order to have the amount of organism used in these mailings. Someone would have noticed this happening."

[23] USAMRIID#17PDFpg 49: FBI summary of Ivins' lab notebooks 14July2004:

"Also contained within the notebook was a Dugway procedure for anthrax spore preparation in bench top fermenters. This standard operating procedure (SOP) draft outlined the culture and processing methods used for preparation of Ba spores in less than 10 liter quantities."

[24] BEI#1 PDFpg 23.

[25] DOJ Amerithrax Investigation Summary, PDF pg 30, summary report pg 26.

[26] USAMRIID#16 pg 76: Dugway Shipments:

5th shipment: 7/21/97 52 ml: 3 x 10^12 total spores
8/2/97 51 ml; 3 x 10^12 total spores

USAMRIID#16 pg 77: Dugway Shipments: [date redacted]

6th shipment: 8/4/97 5 x 10^12 total spores:

USAMRIID#16pg81: Dugway shipments:

7/11/97 60ml: 5 x 10 ^12 total spores
7/17/97 80ml: 3.25 x 10^12 total spores

[27] USAMRIID#16 pg 77: Dugway Shipments: [date redacted]
6th shipment: 8/4/97 5 x 10^12 total spores:
yield apparently 3.8 x 10^12 spores

[28] BEI#2 PDFpg12-13: Ivins interview 31Mar2005:

[29] United States Department of Justice Science Anthrax Press Briefing, Monday August 18, 2008. Transcript accessed 11 April 2010 at:
http://www.fredericknewspost.com/media/pdfs/FBI_0818_afternoon_bfg.pdf

[30] USAMRIID#20 PDFpg 30: 20May2004 Report investigative project re access to post-challenge Ames from Aerobiology Division USARIID:
"Data from aerosol challenges involving Bacillus anthracis (Ba) from the time period August 1998 to Sep 1999 was analyzed to determine potential windows of opportunity for removal of post-challenge Ames during a ten day period following each aerosol challenge. It was estimated based on spore concentrations of material used that any aerosol challenge involving greater than or equal to three animals would constitute enough available Ba to have assemble the anthrax-laced letters."

[31] BEI#1 PDFpg 86: Interview with IVINS 7May2004
"IVINS took the interviewing agents past the autoclave crash door for suite B3, showed how this suite connected to B4, and showed the fermentation room. Two of the fermentors visible in the room have been purchased in the last 3 years or so."

[32] USAMRIID #8 PDFpage 27: interview with USAMRIID employee 3 Oct 21005:
"...Ba Ames has never been fermented at USAMRIID. It would have been too dangerous to have grown Ba Ames in a fermentor."
BEI#2 PDFpg 7-8: Ivins interview 31Mar2005:
"IVINS related that while USAMRIID had fermenters, to his knowledge they were never used to grow any virulent select agents, including Bacillus anthracis. The fermenters were used only to grow non-virulent bacteria, such as Bacillus subtilis. ..."
"...He explained that using a multi-gallon fermenter for this purpose would have been out of the question due to the potentially catastrophic results of an accidental spill of such a large quantity of anthrax. IVINS believed it would be simply too dangerous to produce virulent anthrax in a metropolitan area using a fermenter because of the large quantity yielded."

[33] USAMRIID #5 PDFpage 2: FBI periodic report 29Sep2006:
"Fermentor:
Identification and tracking of fermentors in place at USAMRIID positively identified one fermentor as having been in place in Building [redacted] Room [redacted] around the time of the anthrax mailings. Additional fermentors of interest have been identified for location, materials used, and operator with an undetermined final disposition date. The fermentors ranged in volume from 150 liters to 5-liters. The two largest fermentors were manufactured by New Brunswick Inc, and were not portable. These cold not likely have been used in a discreet manner. The 5-L fermentor also manufactured by New Brunswick was lent to [redacted] by Bruce Ivins. [redacted] indicated the New Brunswick fermentors were likely to predate 2001. Further analysis would be required to determine which fermentors were up and running between 9/11/01 and 10/9/01."
Also see: USAMRIID#3b PDFpage 91-96 09/07/2006 Status Report on Tracking and ID of Fermentors at USAMRIID. For an update on fermenter tracking.

[34] USAMRIID#3b PDFpage 93 09/07/2006 Status Report on Tracking and ID Fermentors:
"20-liter Fermentor: Room [redacted]:
The New Brunswick fermentor, no MMCN Number, no Stock Number was located in [redacted]. Operators included: [redacted] and [redacted]. [redacted] recalled that in 2000, [redacted] run a New Brunswick 20-liter fermentor which involved a vaccine study pertaining to the growth of delta-Ames or delta-Sterne."

[35] BEI#4PDFpg 8: materials recovered from Ivins' residence under search warrant 1Nov2007 included a copy of a USAMRIID hand receipt from 1997 with this entry.

[36] USAMRIID #7 PDFpage 59: Patent # 6,387,665, patent page 5:
"The fermentations describe here were carried out using a New Brunswick Bio-Flo 3000 equipped with a 5.0 liter working volume glass vessel and stainless steel headplate and hemispherical bottom cooling dish. ..."

[37] USAMRIID #7 PDFpage 60: Patent # 6,387,665, patent page 6:
"..fermentations were carried out ... with no pH control or additions other than antifoam C."

[38] Product Information: Silicone Antifoams: Dow Corning Antifoam C:
accessed 11Apr2010 at: http://www3.dowcorning.com/DataFiles/090007c880012087.pdf

[39] USAMRIID #7 PDFpage 58: Patent # 6,387,665, patent page 4.

[40] USAMRIID#8 PDFpg 21: FBI report of 28Sep2005, including review of Laboratory Notebook Review Project:

"On page four of notebook [redacted] Ivins detailed a procedure in which he grew five liters of Ba (strain unknown) to give to [redacted]. None of the rats died after being injected with this preparation and Ivins speculated he had harvested the Ba too early."

[41] USAMRIID#4 PDFpg 105: Interview with a USAMRIID employee 22Feb2008:

"[redacted] also recalled that prior to the Sept 11, 2001 time frame IVINS was USAMRIIDs technical representative to DPG pertaining to a project whereby DPG was contracted out to produce large quantities of wet Ba spores."

[42] USAMRIID#17PDFpg 49: FBI summary of Ivins' lab notebooks 14July2004:

"Also contained within the notebook was a Dugway procedure for anthrax spore preparation in bench top fermenters. This standard operating procedure (SOP) draft outlined the culture and processing methods used for preparation of Ba spores in less than 10 liter quantities."

[43] USAMRIID#4 PDFpage 89, 90: 14Jan2008 interview at Ft Detrick:

"[redacted] advised that the 5 L fermentor, when operational, did not produce a whole lot of noise."

"When using the fermentors, [redacted] recalled that it was standard operating procedures when growing Ba to use anti-foam in order to reduce the buildup of bubbles or foam that would occur during the fermentation process. [redacted] explained that anti-foam would be automatically pumped into the fermentor to reduce the amount of bubbles. [redacted] recalled that the old brand name for anti-foaming agent was called Mazu and believed that Mazu may have been sold out or taken over by a newer company possibly called Sigma."

[44] BASF Technical Bulletin: MAZU DF 204 Defoamer. Accessed 8April2010 at: http://www2.basf.us/businesses/chemicals/performance/pdfs/Mazu_DF_204.pdf

[45] USAMRIID #5 PDFpage 2: FBI periodic report 29Sep2006:

"The 5-L fementor also manufactured by New Brunswick was lent to [redacted] by Bruce Ivins." [MFnote: the name redacted in this quote appears to contain 6 letters].

[46] USAMRIID #5 PDFpage 67: Interview with USAMRIID employee [MF note: from length of redactions, probably male with a 6-letter surname]. 1/8/2007

"[redacted] advised [redacted] spoke to [long redaction] concerning the five (5) liter fermentor. Per [redacted] had previously told an interviewing Postal Inspector that USAMRIID employee BRUCE IVINS lent the 5L fermentor to [redacted] [WFO NOTE: see 279-WF-222936-POI, Serial 1487]. [redacted] clarified [redacted] at the time IVINS lent the fermentor was [long redaction] and not [redacted]. [redacted] did not work for [redacted] until after [redacted] left USAMRIID, circa [redacted]. [redacted reiterated IVINS never lent [redacted] the 5L fermentor, [redacted] has never seen it at USAMRIID, nor does [redacted] maintain any paperwork associated with same."

[47]BEI#2 PDFpg 15-16: 31March2005 Interview with Bruce Ivins:

"IVINS advised that prior to the fall 2001 anthrax attacks, suite B3 contained an extensive number of tubes and flasks containing liquid anthrax spores. When asked how many such containers were stored in the room, he advised there were 'hundreds of containers of all sizes, but probably not thousands.' There was no common labeling scheme or protocol for these containers – they were labeled by the individual researchers to whom they belonged. Each researcher had the discretion to label the containers as he/she saw fit. Many of the containers which held Ames anthrax spores were labeled'Ames.' As an example, IVINS noted the flask containing RMR 1029 was labeled as 'Ames.' There was no inventory of the flasks and tubes. The walk-in refrigerator in B3 contained shelves which were always filled with flasks and tubes of spores."

USAMRIID#8 PDFpg 4: 18Aug2005 Interview with ex-USAMRIID employee:

"[redacted] noted that at [redacted] USAMRIID [redacted] there were no general logs maintained with would show how much organism was grown from the samples, or records to reflect how much of the grown organism was used in testing, and then subsequently destroyed. ..."

[48] Yudhijit Bhattacharjee, "Anthrax Investigation: Silicon Mystery Endures in Solved Anthrax Case" (News of the Week) *Science* vol 327 no 5972 pg 1435, 19Mar2010.

[49] United States Department of Justice Science Anthrax Press Briefing, Monday August 18, 2008. Briefing page 13: Transcript accessed 11 April 2010 at: http://www.fredericknewspost.com/media/pdfs/FBI_0818_afternoon_bfg.pdf

"DR MICHAEL: ...Again, in the letter materials the silicon and oxygen were co-located on the spore coat, within the spore. In fact, we found some vegetative cells that were going through the sporulation process and the spore within the mother cell had this same signature."

[50] Joseph Michael (Sandia National Laboratory), "Microscopy/Weaponization of Bacillus anthracis" presented at: Scientific Review of FBI Anthrax Investigation: Second Committee Meeting, Friday September 25, 2009. Data on percentages of high-silicon spores in this paragraph taken from audio recording of Scientific Review of FBI Anthrax Investigation: Second Committee Meeting, Friday September 25, 2009, Day 2: Part 1: accessed 12Apr2010 at: http://nationalacademies.org/newsroom/nalerts/20090925.html

[51] Joseph Michael (Sandia National Laboratory), "Microscopy/Weaponization of Bacillus anthracis" presented at: Scientific Review of FBI Anthrax Investigation: Second Committee Meeting, Friday September 25, 2009. Discussion of this series of samples is taken from audio recording of: Day 2: Part 1: accessed 12Apr2010 at: http://nationalacademies.org/newsroom/nalerts/20090925.html

[52] BEI#1PDFpg 24: Bruce Ivins interview 12 Feb 2003:

"Double purified material needs to have a chromatogram done to determine whether it was run through a gradient. A trace of the gradient would remain on the material and should be indicated by a peak on the chromatogram. Some possible gradients include sucrose, hypaque, renografin (which became known as renocal but may not be manufactured any longer), or phycoll."

[53] The starting concentration of RMR1029 was 3 x 10^13 spores in 1,000 cc, or 3.0 x 10^10 cfu/ml.

BEI#3 PDFpgs 97-108: Spore Preparation Forms dated Oct 1997 to Apr 2001: Starting concentration for RMR1029 varies from 2.5 to 3.9 x 10^10 cfu/ml.

[54] BEI#1 PDFpg 39: Reference Material Receipt Record entry for 27Aug2001.

[55] BEI#1 DPFpg 7: Ivins interview 31Jan2002:

" Ivins stated that Dugway Proving Grounds in Utah usually makes the spore preparations used in animal challenges at USAMRIID. Ivins maintains a detailed inventory of all these spore production preparations that have been sent from Dugway. All of this inventory is accounted for."

[56] BEI#1 PDFpgs 38,39: Reference Material Receipt Record 22Oct1997 to 18Feb2003

[57] Ref: BEI#1: PDFpg 107-108: Ivins interview 13Jan2005:

"IVINS record of the dissemination of Reference Material Receipt (RMR) 1029, the Ames spores which were a combination of Dugway produced spores and the spores made by IVINS, was kept only for the purpose of allowing the researchers to estimate how much of the material was left so they would not run out of spores for aerosol challenges. The record was not kept as any kind of precise inventory for security reasons. The amounts of remaining material were only estimates and were not accurately measured for each entry."

[58] DOJ Amerithrax Investigation Summary, PDF pg 31, summary report pg 27 footnote 10.

[59] USAMRIID#19 PDFpg 7,8: Ivins interview 8Sep2004:

"IVINS explained that the discrepancies between the volume removed from RMR1029 for the May and June 2001 shipments to Battelle [redacted] and [redacted] respectively) and the volume actually shipped in May and June 2001 [redacted and [redacted] respectively) were due to the fact that IVINS centrifuged the samples and reduced their overall volume prior to shipment to Battelle…."

"When asked about a [redacted] discrepancy in this record, IVINS explained that evaporation over the years as well as math error would account for this missing volume. IVINS stated that RMR 1029 could have lost up to [redacted] a year and this would not be unusual because it is not stored in an air tight container…"

[60] USAMRIID#19 PDFpg 8: Ivins interview 8Sep2004:

"When USAMRIID was getting low on RMR 1029 spores, they contracted Dugway in 2001 to produce additional spores."

[61] DOJ Amerithrax Investigation Summary, PDF pg 32, summary report pg 28 footnote 11.

[62] DOJ Amerithrax Investigation Summary, PDF pg 32, summary report pg 28:

"In fact, the investigation revealed that there were never any additions to RMR1029 after its creation in October 1997."

[63] BEI#2 PDF pgs 29-32: 1Apr2005: Lab Check of Concentration of RMR1029 re Naval Lab discrepancy

[64] BEI#2 PDF pgs 29-32: 1Apr2005: Lab Check of Concentration of RMR1029 re Naval Lab discrepancy

[65] BEI#1 PDFpg 118: telephone call from Ivins 16Feb2005:

"On a separate issue, IVINS verified that he found RMR1029 to be 2.4 x 10^10 cfu/ml and not 2.4 x 10^9 cfu/ml after it was returned to him from FBI custody. IVINS said that the viability was only down 20-30%, not 90%, and that they were able to fix the problem by adding more spores for the aerosol challenge for [redacted]."

[66] BEI#2 PDFpg 14-15: Ivins Interview 31Mar2005:
"IVINS was questioned concerning the viability of RMR 1029 after the FBI returned RMR 1029 to IVNS due to a conflict between his earlier representations that its concentration was 10^10 colony forming units (cfu) per milliliter (ml) and the Navy Medical Research Center's (NMRC's) evaluation that the concentration was 10^9 cfu/ml.

"IVINS stated that the concentration of RMR 1029 was either 2.4 x 10^10 cfu/ml or 2.5 x 10^10 cfu/ml when he checked it after it was returned by the FBI. IVINS advised that he has never observed RMR 1029 drop below approximately 3.5 c 10^10 cfu/ml during its existence. He also said he did not add any more spores to the RMR 1029 material after the FBI returned it to him. When asked whether it would have been possible for someone to take some of the anthrax slurry out of the RMR1029 flask and replace it with water without detection, he responded in the affirmative.

"IVINS could offer no explanation other than "technical error" for the discrepancy between his and NMRC's conflicting measurements of the concentration of RMR 1029. When asked to opine as to what may have caused such drop in viability, IVINS said he did not know. IVINS was highly skeptical of the FBI's claim that the concentration of RMR1029 was really 10^9. He explained that after the FBI gave RMR1029 back to him, he used 25ml for an animal challenge conducted by USAMRIID researcher [redacted]. IVINS said he would not have been able to perform this "animal spray" if the concentration of RMR 1029 had been 10^9 because this concentration would have been too low to challenge the vaccine."

[67] BEI#2 PDF pgs 29-32: 1Apr2005: Lab Check of Concentration of RMR1029 re Naval Lab discrepancy

[68] USAMRIID#20 PDFpg 82-85: 6Jan2005: FBI internal document:
Plans for 'percentage viability' assay to be done on all RMR1029 samples.
BEI#2 PDFpg 88: 6/19/2007: Navy Lab report(? Or old report request?) of conc of RMR1029: Redacted data from 14Feb2005.

[69] BEI#6 PDFpg 39: 6Feb2008: Email to an FBI informant (identity redacted) from IVINS:

"They accuse me of diluting, altering or adulterating an important preparation of anthrax material."

[70] DOJ Amerithrax Summary PDFpg18, summary page 14.

[71] USAMRIID#3b PDFpg 51: Interview with USAMRIID employee 26Apr2006

[72] USAMRIID#4, PDFpg114: Interview with ex-USAMRIID employee 11Apr2008:

"[long redaction] did in fact dry/lyophilize very small amounts of Ba but nothing remotely close to the quantity of Ba powder recovered from the anthrax-laced letter mailings."

[73] USAMRIID#3b PDFpg 50: Interview with USAMRIID employee 26Apr2006:

"[redacted] advised to [redacted] knowledge there was no 'dry work" conducted at USAMRIID, rather it was well known that Battelle was involved in 'dry work.'"

[74] USAMRIID#21 PDFpg 13: Interview 23Jan2007: USAMRIID employee

"Due to [redacted] previous experience with the [long redaction] was originally tasked by [long redaction] believes that [she] worked on this project for about [long redaction] that they had to stop working with dried powder aerosolization due to its duel [sic: FBI misspelling] *use implications."*

"[redacted] worked on this project alone in building 1412 suite [redacted] (only parts of this suite are classified as a containment area)."

Follows heavily redacted passages about duration of study and who was knowledgeable and where lab notebooks should be.

USAMRIID#21 PDFpg 5-6: 16Jan2007 interview with USAMRIID employee.

"When referring to the dry aerosol study that was conducted by the [long redaction] described it as a side project which occurred over a relatively short period of time. [redacted] believed it was [long redaction] explained that [long redaction]. The purpose of this project was to bridge the gap between use of wet aerosolization techniques as the model for studying a disease which in the real world would be caused by a dry aerosol. [redacted] elaborated that if conducting aerosol vaccine challenges using a wet suspension of Ba spores did not accurately reflect a disease (eg inhalational anthrax) caused by a dry aerosol then the results of the model had limited value. The purpose of the bridging study was to demonstrate that a wet aerosol vaccine challenge was a suitable model. [long reaction] USAMRIID [long redaction] was directed by [redacted] to attempt to aerosolize [redaction]. Work performed by [redacted] on this project was conducted in room [redacted] of building [long redaction] designated area at the time."

"[redacted] explained that during this project [redacted] inquired about possibility of using a dried biological agent. However, the administration at USAMRIID denied the use of dried biological agents due to their dual use perception. [long redaction] considered this project just another of [long redaction]."

"[redacted] convey that there were discussions [redacted] about attaining dried Bacillus anthracis (Ba). Discussions detailed that [redaction] could be contracted to provide this material. There was also discussion as to whether or not the material acquired would need to be milled, and if so, from where would a mill be obtained. [redacted] described that the use of dried agents was deemed out of the question by the administration due to its dual use implications."

"[redacted] has no recollection of discussions about dried agents between [long redaction] at USAMRIID. [long redaction] spoke about the dry aerosol project, but to [redacted] knowledge this was the only individual from [redacted] spoke to about this project. [redacted] believes the project was shut down [long redaction]…"

"[redacted] does not recall there being a cease and desist order, however the project died on its own when the word came down that there would be no dried biological agents used. [redacted] admitted that [long redaction] due to the applicability and relevance that this project had with actual threat dynamics."

[75] USAMRIID#1 PDFpg 25: Interview USAMRIID employee 15Feb2005:

"[redacted] concerns about the dried Bg work were not necessarily ethical concerns. [long redaction] The International Convention says that as long as the work is on pathogenesis or vaccine efficacy, the work is not considered offensive in nature"

USAMRIID#21 PDFpg 15: 31Jan2007 interview USAMRIID employee:

"[redaction] a project aimed at evaluating the feasibility of conducting a dry aerosol Bacillus anthracis (Ba) vaccine challenge was initiated. The purpose of the study was to determine the breakthrough point for the recombinant protective antigen (rPA) Ba vaccine, in other words, what concentration of Ba spores would cause the vaccine to fail. Using the wet aerosol systems in place at the time, a high enough concentration of Ba spores could not be achieved. However, generation of a dry aerosol of Ba spores was expected to provide a sufficient concentration of spores thus achieving this breakthrough point. Additionally, a dry aerosol more closely resembles the type of Ba exposure a soldier on the battle field [sic: FBI spelling error] *would likely encounter. For these reasons, [redacted] felt that to demonstrate the true efficacy of the vaccine, they needed to evaluate it against this more applicable threat.*

"[redacted] indicated that the dried aerosol project never got off the ground. Some equipment was purchased and only preliminary experiments were performed using inert dusts or avirulent simulants. The project was ended because [very long redaction] and as a result, the dry aerosol work didn't need to be completed. Discussions about possible sources for dried Ba took place. Specifically, discussion as to whether spores would be produced at USAMRIID or obtained externally through DUGWAY PROVING GROUNDS ensued. [redacted] recalled no discussion with other departments at USAMRIID about producing dried Ba. [long redaction]."

"[long redaction] was good at "playing with equipment" and was interested in trying to develop the capability to generate dry aerosols at USAMRIID. [remaining paragraph redacted]."

"[redacted] dried Bacillus globigii (Bg) from Dugway Proving Ground and indicated that [long redaction] indicated that some preliminary work may have been done with the Bg from Dugway."

[76] BEI#2 PDFpg 5: Ivins Interview 31Mar2005.

[77] BEI#7, PDFpg 132-133: 18Aug2008 Telephone interview with CDC employee re Ivins: Had been contacted previously 27Mar2008 but wanted to add info:

"[redacted] went on to discuss the several methods of drying that were possibly used by IVNS to dry the spores. [redacted] suggested acetone drying as a possibility. Acetone drying was a common practice at Dugway Proving Ground (DPG). IVINS, having worked closely with researchers at DPG, would have had knowledge of this practice."